

NATIONAL HUMAN GENOME RESEARCH INSTITUTE

Division of Intramural Research

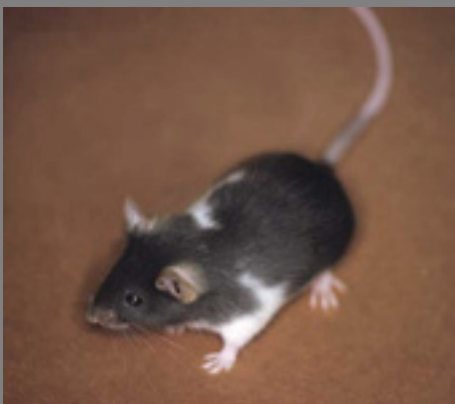


## *The Rich Resources of Mouse Genetics*

*Dawn Watkins-Chow, Ph.D.  
Genetic Disease Research Branch*

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | [genome.gov/DIR](http://genome.gov/DIR)





# *Outline*

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- History of Mouse Genetics
- The Mouse Genome
- Reverse Genetics: Engineered Mice
- Forward Genetics: Screens for Induced Mutations
- Strategic Breeding for Diversity
- Public Database Resources

# *History of the Laboratory Mouse*

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- 1100 BC “Fancy” mouse breeding of coat color variants in Asia & Europe
- 1900 Retired schoolteacher Abbie Lathrop breeds fancy mice in Granby, MA
- 1909 first inbred strains established
- 1929 The Jackson Laboratory founded
- 1962 nude mouse
- 1980 first transgenic mouse
- 1989 first knockout mouse
- 1990 explosion of engineered mice & beginning of mouse genome project



# *Mouse Stocks*

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- Outbred Stocks

- Genetically undefined, bred to maintain heterozygosity
- Cheap & healthy for when genotype does not matter
- Should not be used for breeding experiments

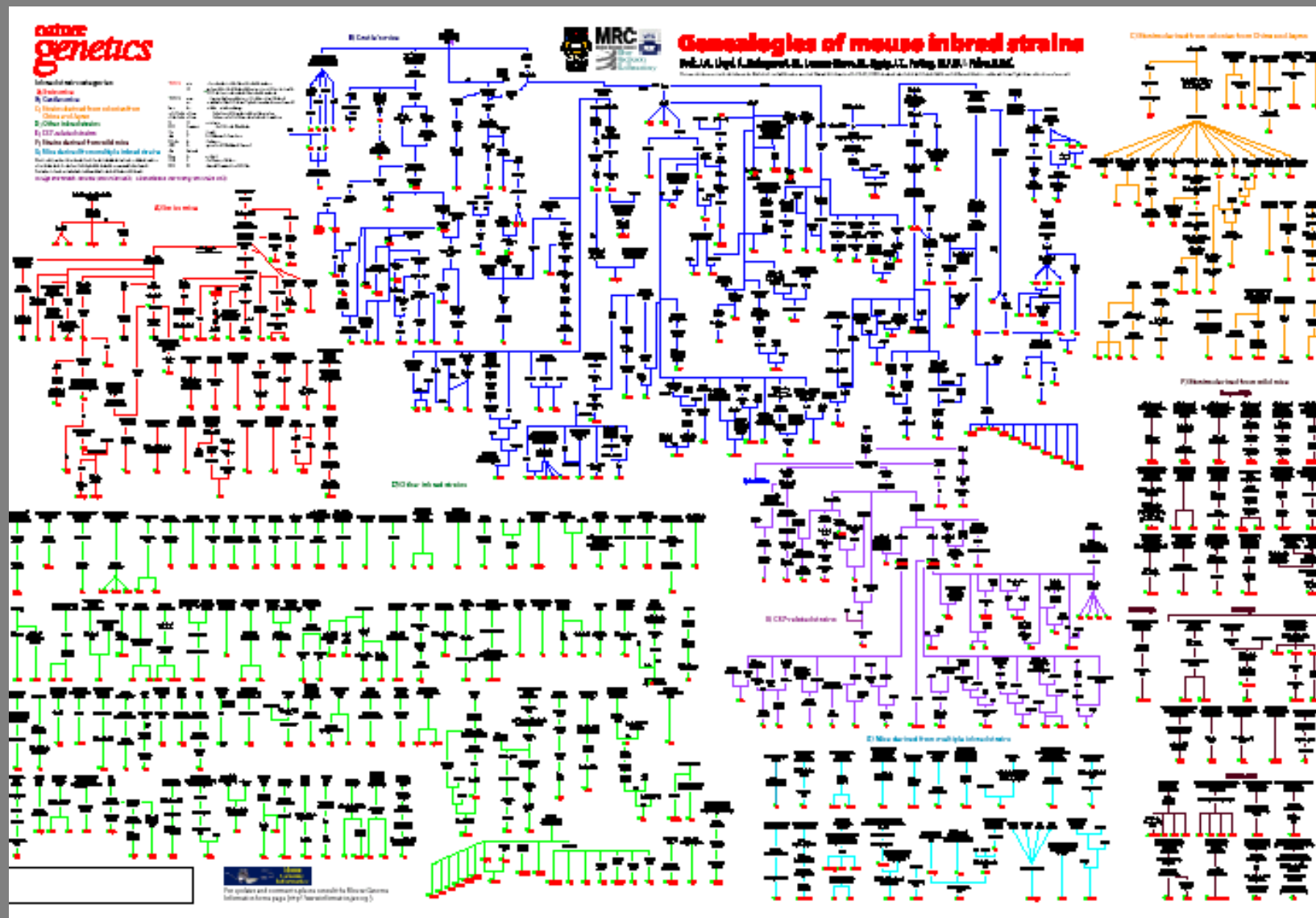
- Inbred Strains

- Bred for homozygosity (>100 strains available)

- Several commercial vendors available

- JAX ([jaxmice.jax.org](http://jaxmice.jax.org))
- Taconic ([www.taconic.com](http://www.taconic.com))
- Charles River ([www.criver.com](http://www.criver.com))

## *Inbred Strain Genealogies*



*Nature Genetics* **24:23-25** (2000)

# Mouse Phenome Database

**Mouse Phenome Database**

[Your flagged measurements](#)

[Home](#) [Contact](#) [About](#) [FAQ](#) [News](#) [SNPs](#) [Downloads](#) [Preferences](#)

MPD search:

All phenotype strain survey data > blood chemistry > glucose

[Plots & tools wizard](#)

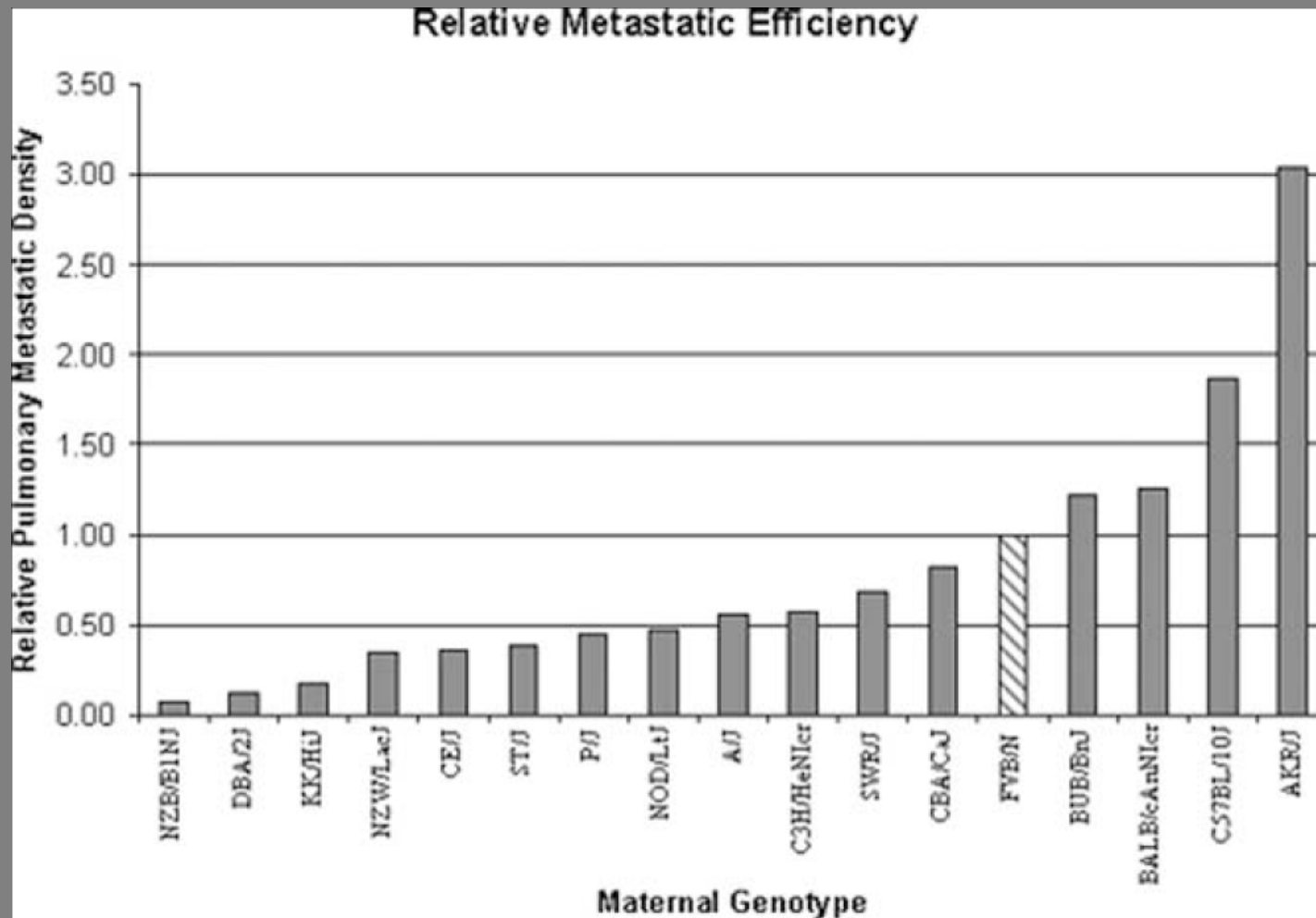
[Change list style](#)
[Display strain values](#)
[Compare 2 strains](#)
[Specific strain panels](#)

## blood chemistry — glucose

Eumorphia1	glucose (plasma GLU) [mg/dL]	6 strains age 12wks
Jaxpheno3	glucose (plasma GLU, 4hr fast) [mg/dL] • age 8wks • age 16wks <a href="#">age comparison</a>	11 strains ages: see details
Naggert1	glucose (plasma GLU, 4hr fast) [mg/dL] baseline and high-fat diet 17wks <ul style="list-style-type: none"> <li>• baseline</li> <li>• after 17wks on diet</li> <li>• Δchange after diet</li> </ul> <a href="#">diet effect comparison</a>	43 strains age 7-9wks at baseline
Lake1 B6.A consomic	glucose (plasma GLU, 4hr fast) [mg/dL]	23 strains age 8wks
Svenson2 B6.PWD consomic	glucose (plasma GLU, 4hr fast) [mg/dL]	18 strains age 8wks
QTL-Reifsnyder1 BC1 (NON   NZO)	QTL analysis data set: Body weight, composition, glucose, insulin and leptin	N=204 age 4-24wks ♂
QTL-Smith1 F2 (B6   CAST)	QTL analysis: Fat, carbohydrate, and calorie intake 2-choice macronutrient diet 10d	N=502 age 9-13wks ♂

<http://phenome.jax.org/pub-cgi/phenome/mpdcgi>

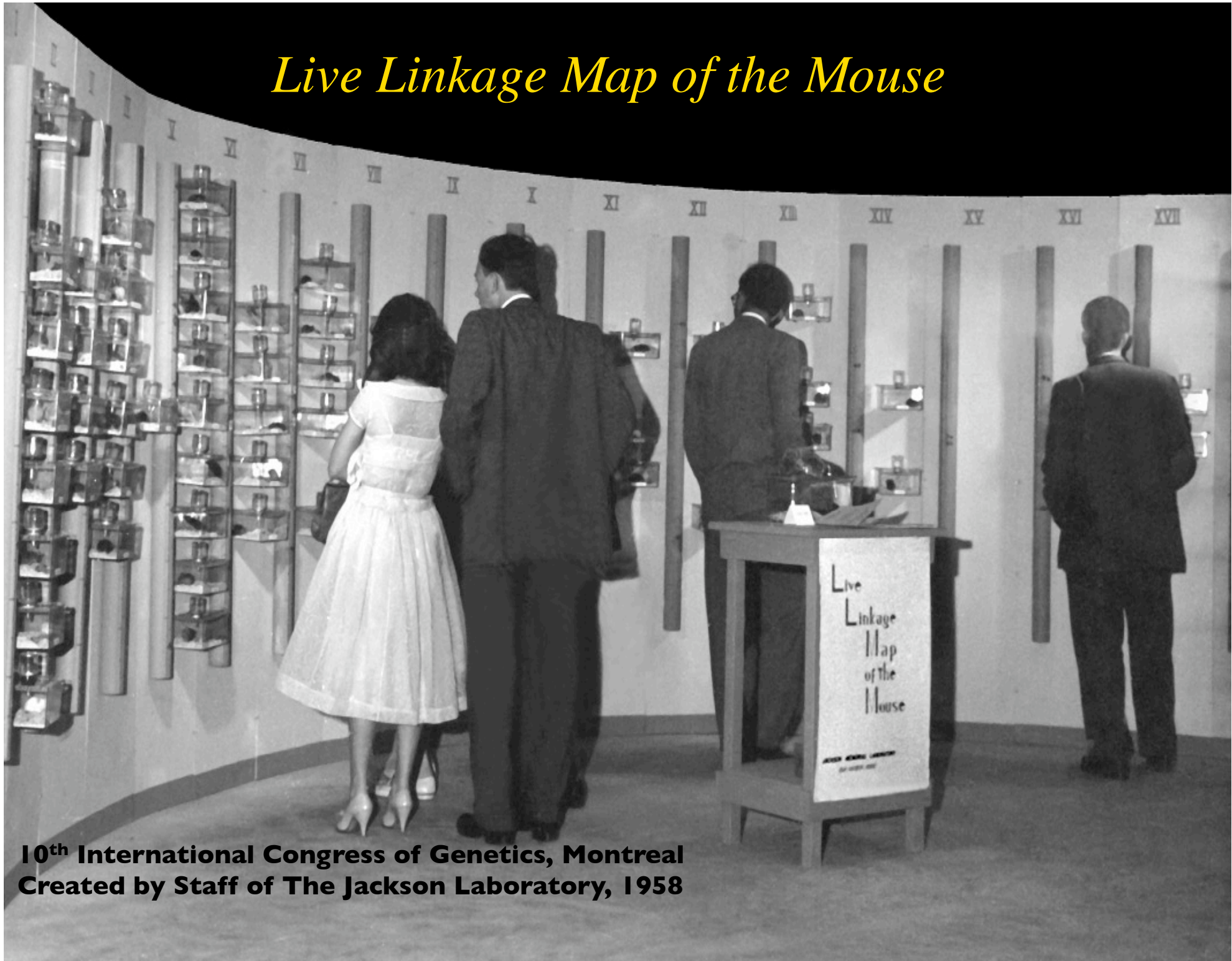
## *Affect of Background Strain*



T. Lifsted et al. *Int. J. Cancer* (1998) 77:640-644

K. Hunter. *Canc Letter* (2003) 200:97-105

# *Live Linkage Map of the Mouse*



**10<sup>th</sup> International Congress of Genetics, Montreal  
Created by Staff of The Jackson Laboratory, 1958**



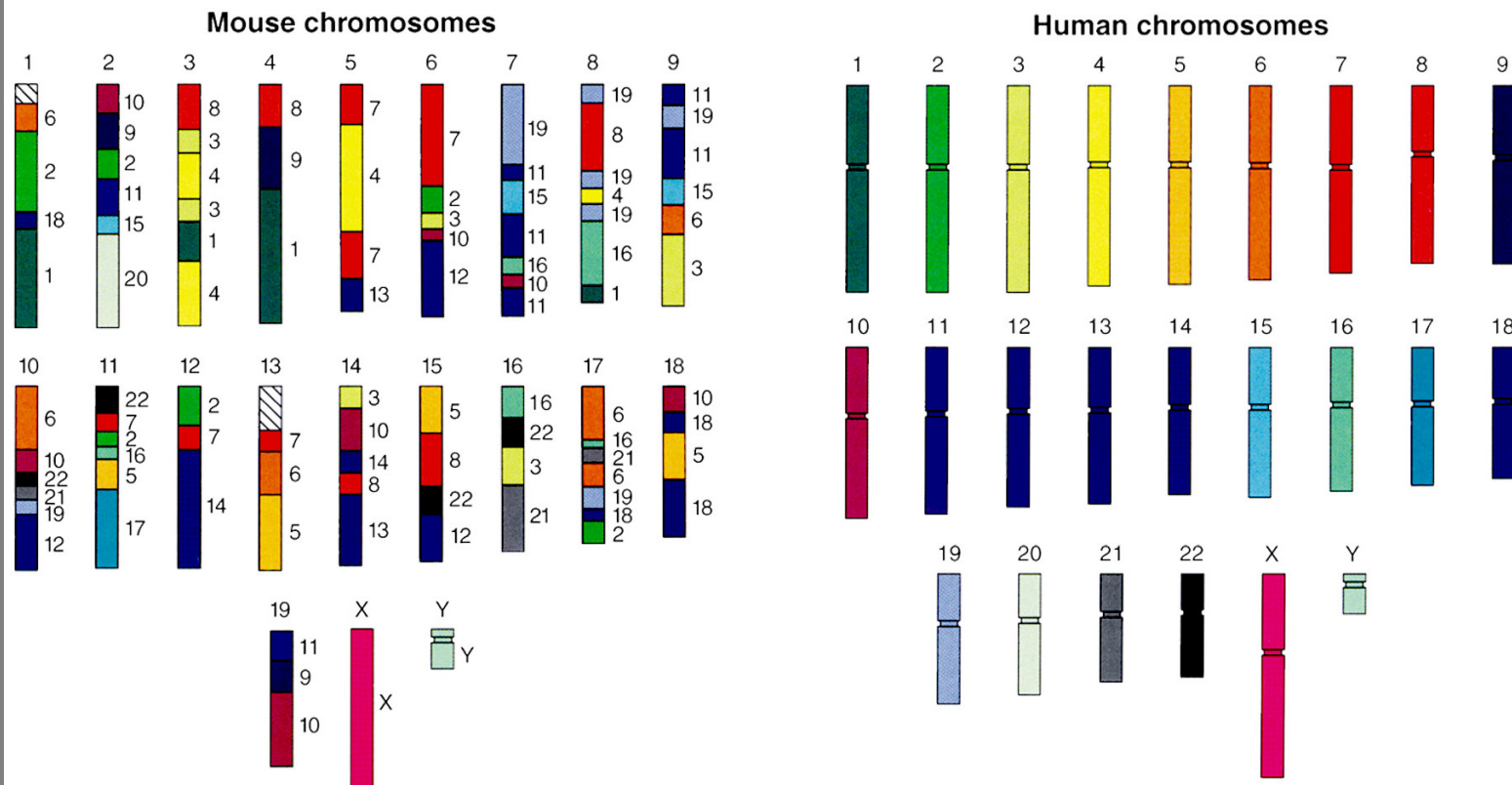
# The Mouse Genome



Nature 420:520-562 (2002)

# Synteny

## Mouse and Human Genetic Similarities



Courtesy Lisa Stubbs  
Oak Ridge National Laboratory

# *The Mouse Genome*

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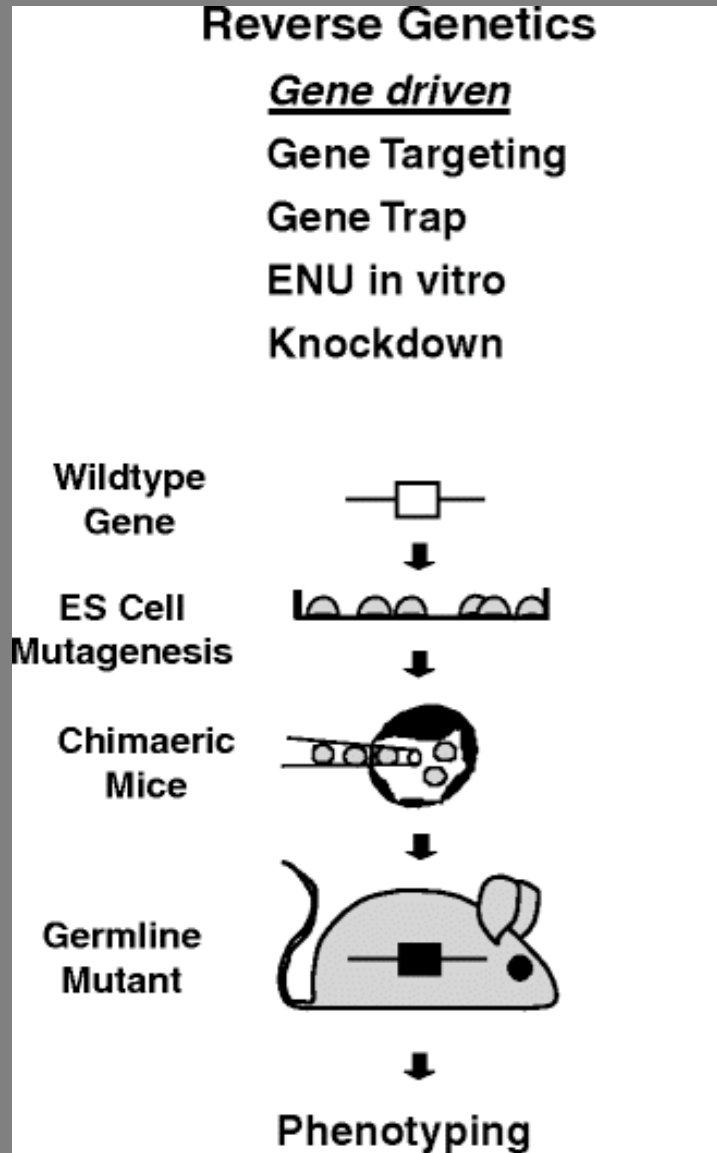
- 19 autosomes, XY
- NCBI Build 37
  - $2.66 \times 10^9$  bp
    - 124 contigs mostly >5000 kb
  - 28,278 transcripts
  - C57BL/6J is reference strain
- Web browsers
  - <http://genome.ucsc.edu>
  - <http://www.ensembl.org>
  - <http://www.ncbi.nlm.nih.gov>
    - Includes alternate partial assemblies

# The Mouse Genome



<http://www.ncbi.nlm.nih.gov/projects/mapview/>

# *Strategies to identify gene function*



From: Gene Knockout Protocols



# *Large Scale Efforts*



## **International Knockout Mouse Consortium**

IKMC Gene Progress Summary

Total Genes	KOMP		EUCOMM	NorCOMM	TIGM
	CSD	Regeneron			
Project goal	5000	3500	8000	500	-
Vectors generated	5099	3295	4477	312	-
Vectors available	4791	2296	4477	312	-
ES cells generated	2650	1808	2386	45	-
ES cells available	1940	1136	2386	45	10699
Mutant mice generated	168	160	279	0	-
Mutant mice available	168	71	279	0	-

<http://www.knockoutmouse.org>

# Large Scale Efforts



International Knockout Mouse Consortium

**Search** **Browse** [help](#)

Search IKMC database  
Enter gene symbols, gene IDs or genome location

e.g., Adam19, Pax, ENSMUSG00000020681,  
Chr13:22210730-22311689

[Home](#) [About IKMC](#) [Download](#) [BioMart](#) [Nominate gene](#) [FAQ](#) [Order Products](#) [Contact IKMC](#)

## About...

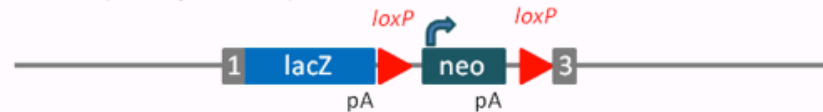
- IKMC
  - IKMC
  - Targeting strategies
  - IKMC alleles
- I-DCC
- KOMP
  - EUCOMM
  - NorCOMM
  - TIGM

[Home](#) » [View all IKMC allele types](#)

## View all IKMC allele types

### Allele type:

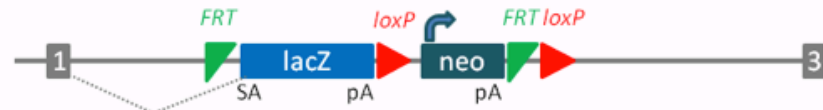
Deletion (lacZ replacement)



### Pipeline:

KOMP-REGN

Deletion (lacZ-tagged)



KOMP-CSD

Deletion (lacZ-tagged, conditional-ready)



NorCOMM


<http://www.knockoutmouse.org>

# Finding the Mice you Need

IMSR Summary										
<p><b>Query Summary</b>            State equals live mouse            Gene/Allele Symbol/Name contains RAS searching Current/Synonyms            Display Limit equals 10000</p>										
281 matching items displayed										
+ Name carries approved nomenclature. - Name does not carry approved nomenclature. ? Name has not been reviewed for nomenclature.										
N	Strain/Stock Designation	Holder Site	State	Strain Type(s)	Strain/Stock Synonyms	Chr	Allele Symbol	Allele Name	Gene Name	Mutation Type(s)
+	<a href="#">129-Alp<sup>tm1(cre)Nagy</sup>/J</a>	JAX	live	coisogenic strain		4	<a href="#">Alp<sup>tm1(cre)Nagy</sup></a>	targeted mutation 1, Andras Nagy	alkaline phosphatase, liver/bone/kidney	recombinase(cre/flip)
						10	<a href="#">Cdh23<sup>ahl</sup></a>	age related hearing loss 1	cadherin 23 (otocadherin)	
+	<a href="#">129P1/ReJ</a>	JAX	live	inbred strain		18	<a href="#">Polr<sup>d</sup></a>	polymerase iota deficient	polymerase (DNA directed), iota	spontaneous mutation
						8	<a href="#">Disc1<sup>del</sup></a>	deletion	disrupted in schizophrenia 1	spontaneous mutation
+	<a href="#">129P3/J</a>	JAX	live	inbred strain, segregating inbred	129/J	10	<a href="#">Cdh23<sup>ahl</sup></a>	age related hearing loss 1	cadherin 23 (otocadherin)	
						5	<a href="#">Rmcf<sup>d</sup></a>	MCF resistant	resistance to MCF virus	spontaneous mutation
						8	<a href="#">Disc1<sup>del</sup></a>	deletion	disrupted in schizophrenia 1	spontaneous mutation
						18	<a href="#">Polr<sup>d</sup></a>	polymerase iota deficient	polymerase (DNA directed), iota	spontaneous mutation
+	<a href="#">129S-Parp1<sup>tm1Zqw</sup>/J</a>	JAX	live	mutant strain	129S-Adprt1 <sup>tm1Zqw</sup> /J, 129S-Aprt <sup>tm1Zqw</sup> , 129S-Aprt <sup>tm1Zqw</sup>	1	<a href="#">Parp1<sup>tm1Zqw</sup></a>	targeted mutation 1, Zhao-Qi Wang	poly (ADP-ribose) polymerase family, member 1	targeted mutation
+	<a href="#">129S-Top2b<sup>tm2Jcw</sup>/J</a>	JAX	live	coisogenic strain		14	<a href="#">Top2b<sup>tm2Jcw</sup></a>	targeted mutation 2, James C Wang	topoisomerase (DNA) II beta	targeted mutation
+	<a href="#">129S6.CB(B6)-Del(1)1Brk Gpi1<sup>b</sup>/Gpi1<sup>c</sup>/BrkMdfJ</a>	JAX	live	congenic strain, mutant strain	129S6.CBA(B6)-Del(1)1Brk Gpi1 <sup>b</sup> /Gpi1 <sup>c</sup> /BrkMdfJ	1	<a href="#">Del(1)1Brk</a>	deletion, Chr 1, Jane Barker 1	deletion, Chr 1, Jane Barker 1	chromosomal aberration
						7	<a href="#">Gpi1<sup>b</sup></a>	b variant	glucose phosphate isomerase 1	

<http://www.findmice.org>


# Database Resources

 [Tour our new Quick Search](#)

## Mouse Genome Informatics

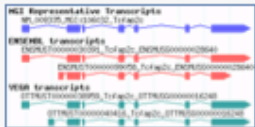
[About](#) [Help](#) [FAQ](#)

[Search](#) [Download](#) [More Resources](#) [Submit Data](#) [Find Mice \(IMSR\)](#) [Contact Us](#)


  [Quick Search](#)

[Explore MGI](#) [All Search Tools](#)

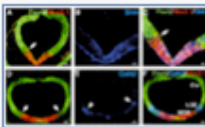
**Genes**



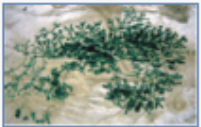
**Phenotypes**




**Expression**



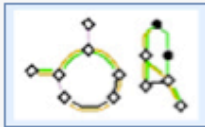
**Recombinases (cre)**



**Function**




**Pathways**



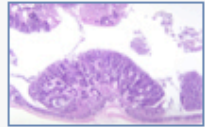
**Strains / SNPs**

Variation Type	DBP/2J	PJ/NU	MDL/ELJ	Allele Summary (all strains)
SNP	G	G	A	A/G
SNP	C	C	T	C/T

**Orthology**




**Tumors**



Done

<http://www.informatics.jax.org/>

# Sharing the Mice you Have



MMRRC  
Mutant Mouse  
Regional Resource Centers  
supported by NCRR-NIH

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About MMRRCCatalog and DistributionSubmissionsSite Map

Start SubmissionInstructionsOverviewSpecial ArrangementsDonor MTA

## MMRRC Submission Application

### Strain Submission Terms and Conditions

As the Donating Investigator, you are asked to agree to and meet the following terms and conditions for any strain submitted to the MMRRC:

1. I confirm that my mouse strain was created and maintained under an approved Institutional Animal Care and Utilization Committee (IACUC) protocol.
2. If this strain is accepted, I agree, in a timely fashion, to:
  - a. provide a detailed genotyping protocol;
  - b. complete and submit the standard MMRRC Donor Material Transfer Agreement (see [sample Donor MTA](#)) for my strain, which details the formal agreement between me, my institution and the MMRRC for preservation and redistribution of my strain;
  - c. provide a recent and institutionally certified health status report in advance of shipping this mouse strain to the assigned facility;
  - d. provide this strain, **within six months**, in a single shipment of live mice or cryopreserved materials in the form and quantities that I have agreed to provide to the MMRRC facility (see [Transfer Methods](#) for additional information);
  - e. take responsibility for the cost of preparation and shipment of my mouse strain to the assigned facility.
3. If I transfer my strain via cryopreserved embryos or germplasm, I accept the risk that it may not be possible to resuscitate my strain. I understand that the MMRRC will make a reasonable effort at resuscitation. I will not hold the MMRRC responsible for the loss of my strain.
4. I accept that the MMRRC will maintain my strain as appropriate for the overall benefit of the entire strain repository resource, and that my strain may be maintained as a cryopreserved-only archive if demand is inadequate to justify maintenance of a breeding colony.
5. I acknowledge that the MMRRC is not liable and will not reimburse for expenses incurred in the event that availability of this strain is further restricted subsequent to its acceptance due to ownership or distribution rights of third parties.

Please indicate that you have read, accept and can meet the above terms and conditions.

I Accept

<http://mymouse.org>

<http://www.mmrrc.org>

## [Screen my mouse for specific disorders](#)

Contact a mouse phenotyping laboratory

### [I have a mouse](#)

List your mutant now (this takes 1 minute)

### [I am looking for a mouse](#)

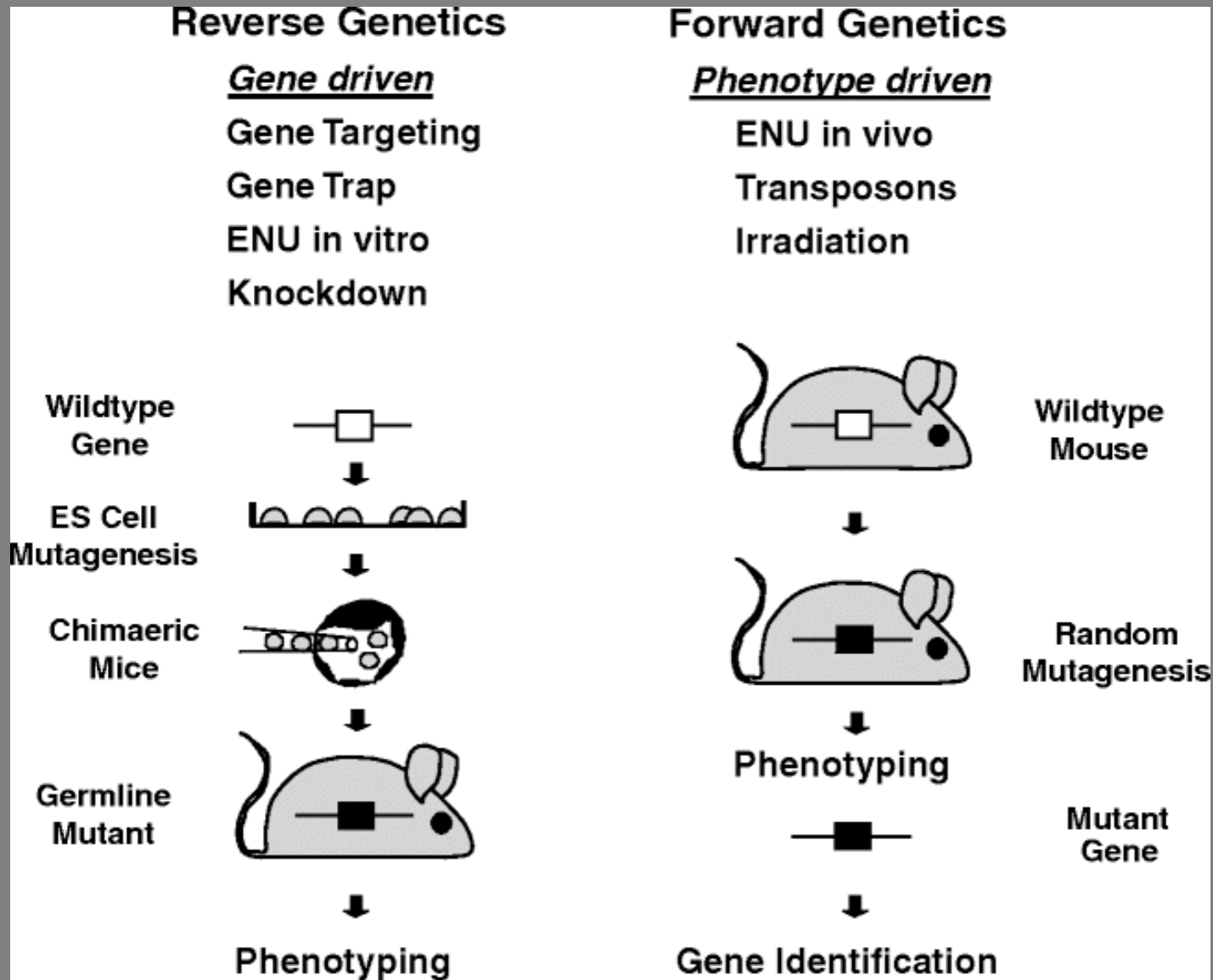
Find one here, or go directly to the  
[complete list of available mice](#)

### [Notify me](#)

Be informed when new mutants and new screens become available



# Strategies to identify gene function



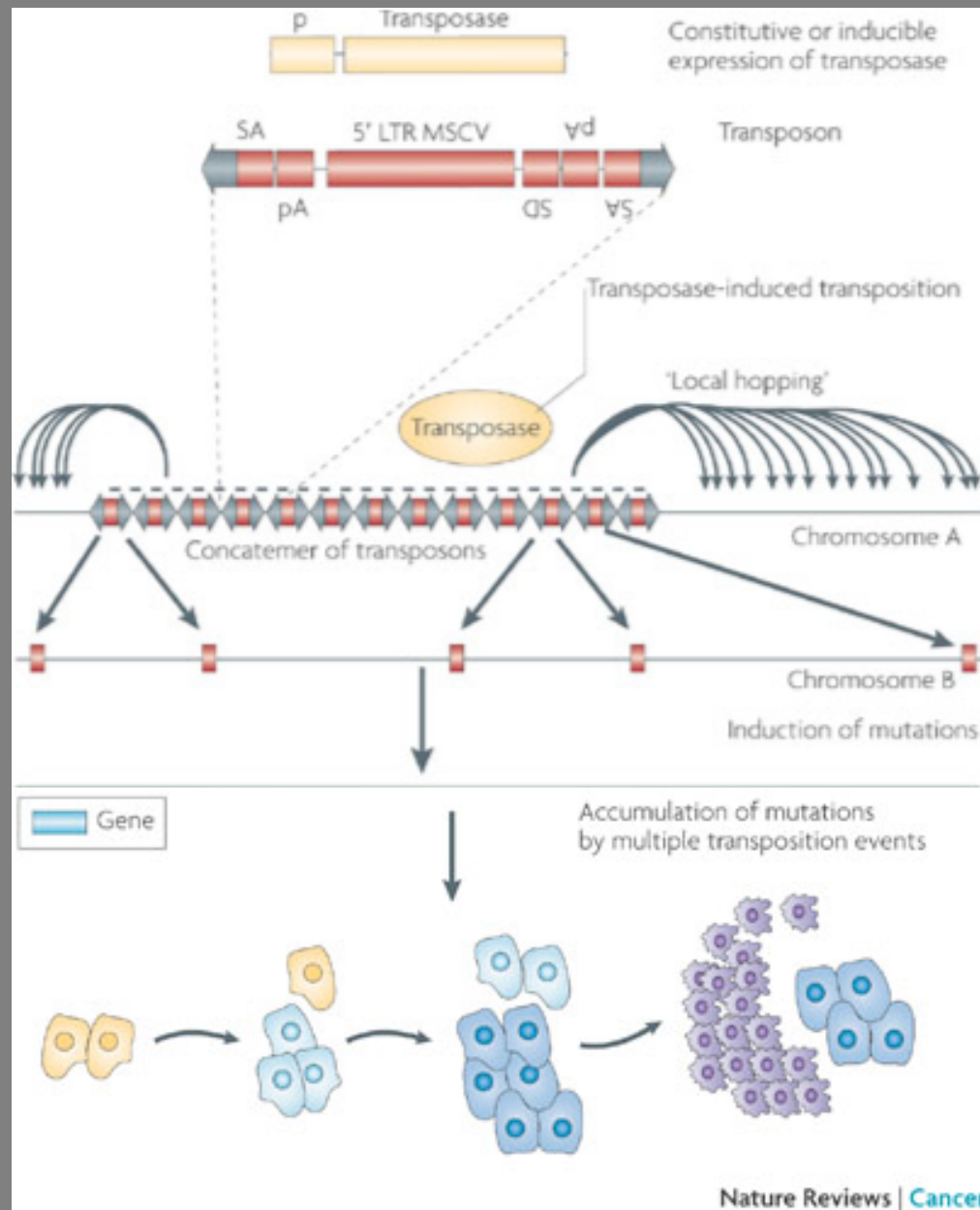
From: Gene Knockout Protocols

# *Forward Genetics*

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- *Retroviral Insertional Mutagenesis*
  - *MMTV, MuLV*
- *Transposon Mediated Mutagenesis*
- *ENU Mutagenesis*

# Transposon Mediated Mutagenesis



Kool & Berns  
*Nature Reviews Cancer*  
(2009) 9:389-399.

# *Transposon Mediated Mutagenesis*

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- Sleeping beauty

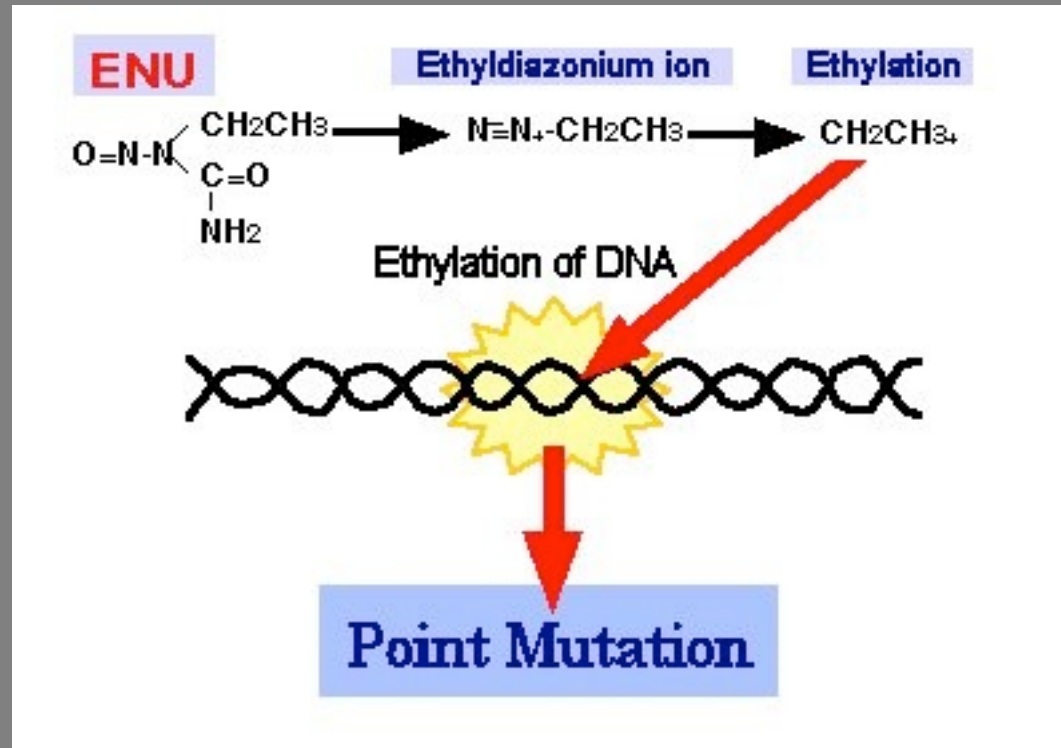
Keng et al. (2005) *Nature Methods* **2**: 763-769

- piggyBac

Ding et al. (2005) *Cell* **122**: 473-483

# *ENU Mutagenesis*

- ENU mutagenesis (ethylnitrosourea)



- Alkylating agent
- Mutations introduced into spermatogonial stem cells



# *ENU Mutagenesis Large Scale Screens*

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**Riken**

**broad phenotyping**

**MRC Harwell**

**broad phenotyping**

**The Jackson Laboratory**

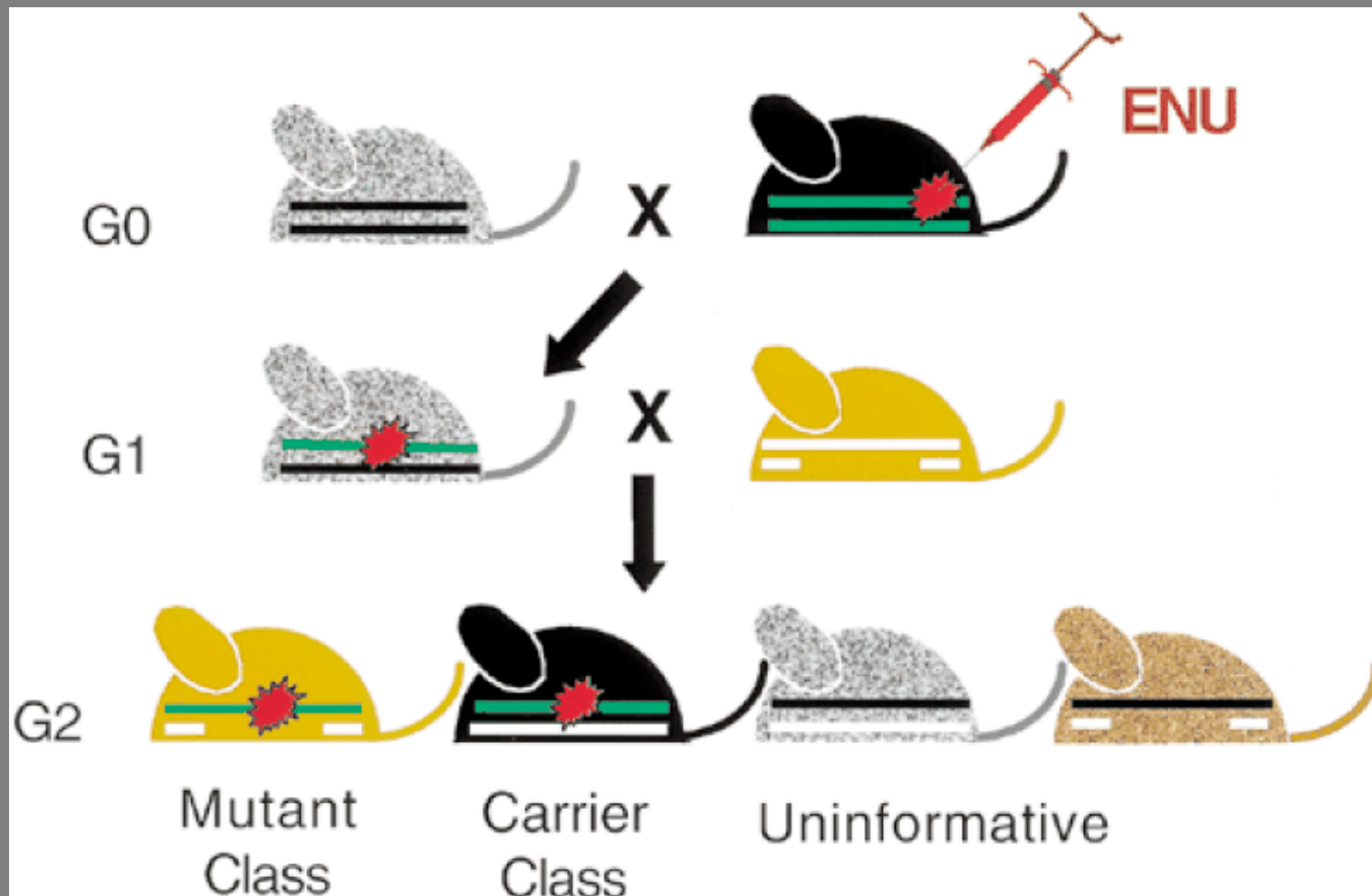
**Neurological, reproductive, heart, lung, blood**

**Baylor College of Medicine**

**Chromosome 11**

# *ENU Mutagenesis*

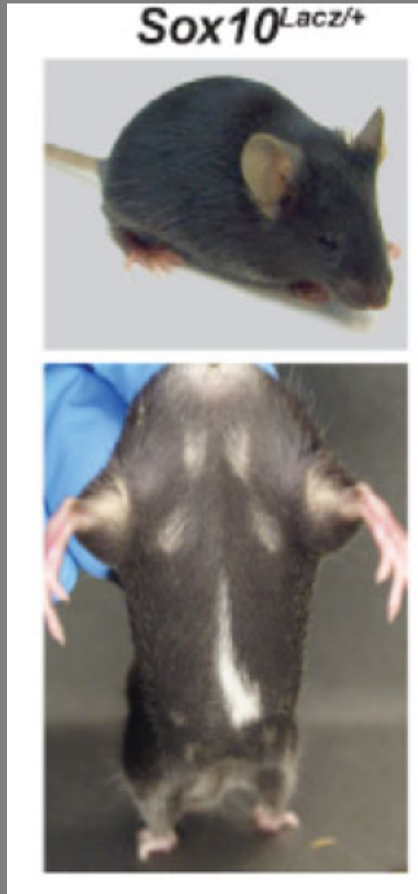
## Region specific screens



# *ENU Mutagenesis*

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## Sensitized/Modifier screens

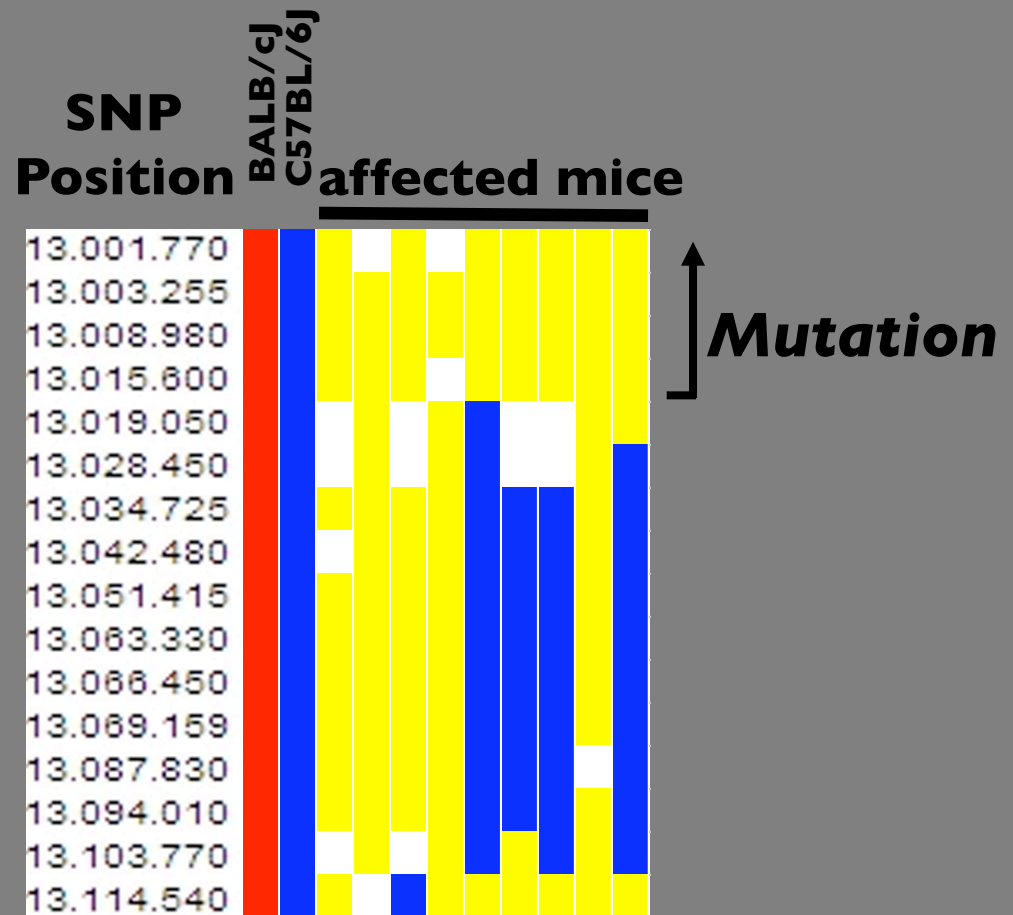


Matera et al. Hum Mol Gen (2008) 17:2118-2131

# Mapping Phenotypes

SNP or SSLP markers polymorphic between the parental mouse strains (C57BL/6J and BALB/cJ) are used to genotype affected mice throughout the genome

- Homozygous BALB/cJ
- Homozygous C57BL/6J
- Heterozygote
- No call



# *Polymorphic Variation*

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- RFLP
- Simple sequence length polymorphism (SSLP)
  - SSRP, STRP, Dinucleotide repeat, microsatellites
- Copy number variation (CNVs)
- Single Nucleotide Polymorphisms
  - Over 10,000,000 SNPs in NCBI dbSNP
  - From 86 strains

# Searching for Polymorphic Variation

- <http://phenome.jax.org> & <http://www.informatics.jax.org>
- 167 SNPs polymorphic between B6 and 129 within 2 kb of Cftr

**MGI** [Tour our new Quick Search](#)  [Quick Search](#)

[About](#) [Help](#) [FAQ](#) [Home](#) [Genes](#) [Phenotypes](#) [Expression](#) [Recombinases](#) [Function](#) [Pathways](#) [Strains / SNPs](#) [Orthology](#)

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## Mouse SNPs

Query Results -- Summary

**You searched for...**  
**Selected Strain(s):** equals **129S1/SvImJ**  
**Reference Strain:** equals **C57BL/6J**  
**SNPs Returned:** equals **different from Reference Strain**  
**Gene Symbol/Name:** equals **Cftr** searching current symbols.  
**Include SNPs located:** **within 2 kb** of genes specified by Gene Symbol/Name  
**Display Limit:** equals **1000**  
**Allele Display Mode:** equals **nucleotide values**

167 matching SNPs displayed

**Legend**  
*Results are sorted by chromosome/coordinate value. Chromosome transitions are marked by blank rows.*

SNP ID (dbSNP Build 128)	Map Position (NCBI Build 37)	rs orient	Gene : dbSNP Function Class	Assays (ss)	Variation Type	C57BL/6J	129S1/SvImJ	Allele Summary (all strains)
rs3157349 <a href="#">MPD</a>   <a href="#">dbSNP</a>   <a href="#">MGI SNP Detail</a>	Chr6:18122723	f	<a href="#">Cftr</a> : Intron	<a href="#">2</a>	SNP	A	C	A/C
rs3161305 <a href="#">MPD</a>   <a href="#">dbSNP</a>   <a href="#">MGI SNP Detail</a>	Chr6:18122761	f	<a href="#">Cftr</a> : Intron	<a href="#">2</a>	SNP	T	A	A/T
rs3157350 <a href="#">MPD</a>   <a href="#">dbSNP</a>   <a href="#">MGI SNP Detail</a>	Chr6:18122824	f	<a href="#">Cftr</a> : Intron	<a href="#">2</a>	SNP	A	C	A/C

Done

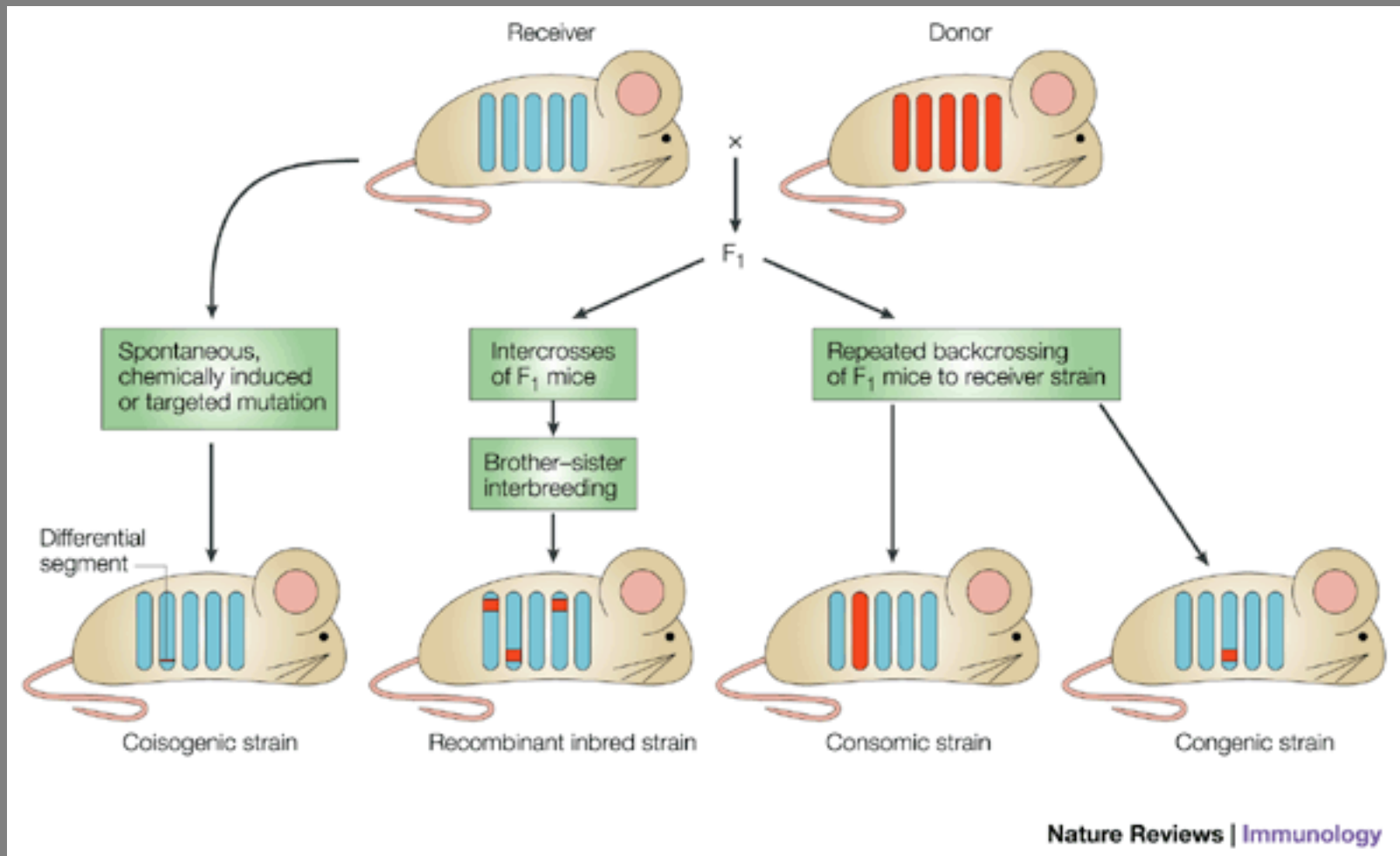


## *Standard Crosses*

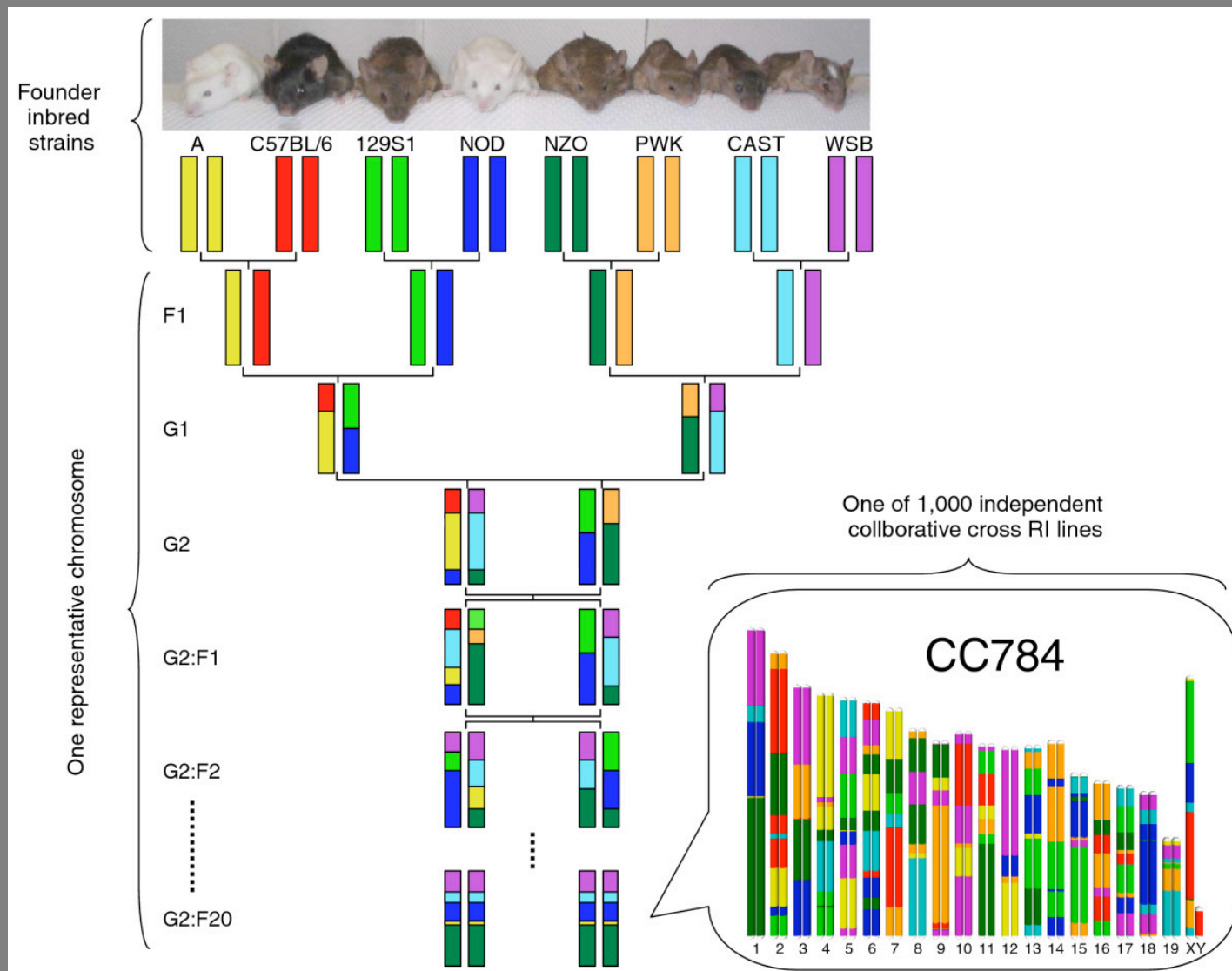
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- Incross: mating between individuals of an inbred strain.  
Used for inbred strain maintenance.
- Outcross: mating between 2 unrelated strains. To generate F1s.
- Backcross: mating between a heterozygous F1 and one of the parent strains. Used in linkage analysis.
- Intercross: mating between two identically heterozygous individuals. Used in linkage analysis.

# Designer Crosses



# Collaborative Cross



## *Reference Material*

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- *Mouse Genetics, Concepts and Applications* (1995) Silver, L.M. Oxford University Press, New York, NY
- *Genetic Variants and Strains of the Laboratory Mouse*, 3rd Edition (1996) Edited by M. F. Lyon, S. Rastan and S.D.M. Brown. Oxford University Press, New York, NY
- **Online Books:**  
[http://www.informatics.jax.org/resources.shtml#res\\_books](http://www.informatics.jax.org/resources.shtml#res_books)
- **Online Resources:**  
<http://www.informatics.jax.org/resources.shtml>

